A Genomic Approach to Elucidate the Molecular Mechanisms Underlying Manganese Neurotoxicity

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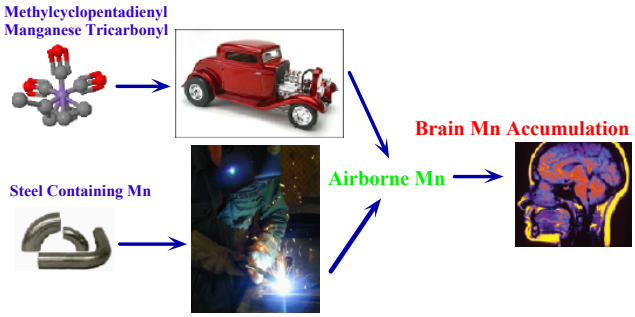
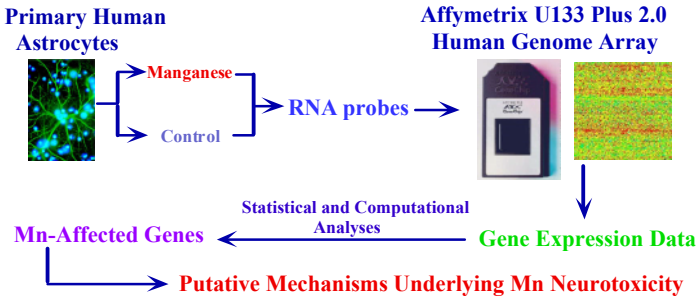
1. Human Exposure to Manganese May Cause Manganism: A Parkinsonian-Like Disorder



Environmental exposure to manganese can lead to accumulation of manganese in the brain. Human exposure to manganese can occur through various environmental sources. The recent addition of the manganese-containing compound methylcyclopentadienyl manganese tricarbonyl to gasoline as an anti-knock agent has raised concern regarding exposure to airborne manganese as a public health issue. In occupational settings such as those present in welding factories, workers may be exposed to elevated levels of manganese.

2. The Application of Genomic Technology to Study Mechanisms of Manganese Neurotoxicity



3. Manganese can alter the expression of a subset of genes with various distinct functions

Tot (annotated)	Cyto	Cell	Transp	Dev	Sign Trans	Trans Reg
734 (316)	< 414+ 320-	30< 28+ 2-	15-	36< 18+ 18-	38< 25+ 13-	41< 26+ 15-	30< 13+ 17-

*The up-regulated and down-regulated genes in primary human astrocytes (Figure 1) were categorized by using the NIH DAVID annotation program and the Gene Ontology algorithm in GeneSpring. Listed here are the numbers of altered genes encoding cytokine and inflammatory functions (Cyto), regulators of cell cycle and DNA replication and repair (Cell), transporters (Transp), transcriptional regulators (Trans Reg) and signal transducers (Sign Trans), and those with developmental-relevant functions (Dev). The total (Tot) number of altered transcripts by Mn is also shown; the number of annotated(classified) genes is shown in parentheses. "+" indicates upregulated genes while "-" indicates down-regulated genes

4. Manganese interferes with cell cycle progression and causes astrocytes to accumulate in S phase

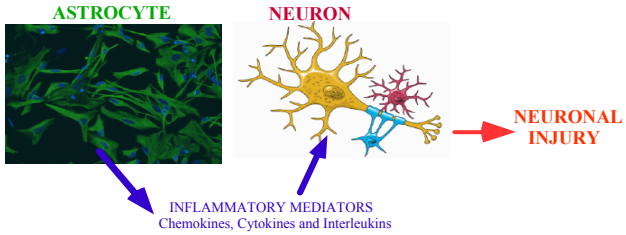
	Control	Mn-treated	p-value
G1	$55.8^{\pm 0.9}$	$36.7^{\pm 1.3}$	0.0003
S	$36.3^{\pm 1.1}$	$54.9^{\pm 1.3}$	0.0003
G2	$3.8^{\pm 0.7}$	$4.5^{\pm 0.2}$	0.57

*Primary human astrocytes treated with no reagent (control) or 200 mM MnCl2 (Mn-treated) for 7 days were subjected to flow cytometric analysis as described in Materials and Methods. The shown data are averages from three experiments, and the two-tailed t-test p-value was calculated by using the SAS software

5. Manganese enhances the expression of a group of genes encoding cytokines and inflammatory functions

Gene Name	Fold Change	Common	Genbank	Description
202912_at	3.8	ADM	NM_001124	adrenomedullin
221009_s_at	10	ANGPTL4	NM_016109	angiopoietin-like 4
223333_s_at			AF169312	
208075_s_at	3.1	CCL7	NM_006273	chemokine (C-C motif) ligand 7
233137_at	4.2	CD48	AF143887	CD48 antigen (B-cell membrane protein)
1553562_at	5.1	CD8B1	NM_172100	CD8 antigen, beta polypeptide 1 (p37)
208488_s_at	4.2	CR1	NM_000651	complement component (3b/4b) receptor 1, including Knops blood group system
237038_at	2.4	CXCL14	AI927990	chemokine (C-X-C motif) ligand 14
218002_s_at			NM_004887	
222484_s_at			AF144103	
209774_x_at	4.1	CXCL2	M57731	chemokine (C-X-C motif) ligand 2
204470_at			NM_001511	
207850_at	4.7	CXCL3	NM_002090	chemokine (C-X-C motif) ligand 3
206336_at	2.4	CXCL6	NM_002993	chemokine (C-X-C motif) ligand 6
224239_at	3.2	DEFB103	AF301470	defensin, beta 103
203717_at	2.8	DPP4	NM_001935	dipeptidylpeptidase 4
205767_at	8.8	EREG	NM_001432	epiregulin
221577_x_at	2.7	GDF15	AF003934	growth differentiation factor 15
209728_at	3.5	HLA-DRB4	BC005312	major histocompatibility complex, class II, DR beta 3
207160_at	4.2	IL12A	NM_000882	interleukin 12A
206693_at	2.5	IL7	NM_000880	interleukin 7
1554544_a_at	2.4	MBP	L18865	CDNA clone MGC:70813
206560_s_at	3.6	MIA	NM_006533	melanoma inhibitory activity
209652_s_at	2.9	PGF	BC001422	placental growth factor
204748_at	2.7	PTGS2	NM_000963	prostaglandin-endoperoxide synthase 2
206157_at	2.3	PTX3	NM_002852	pentaxin-related gene, rapidly induced by IL-1 beta
212171_x_at	2.0	VEGF	H95344	vascular endothelial growth factor
211527_x_at			M27281	

7. Cartoon Illustrating How the Expression of Inflammatory Mediators by Astrocytes in Response to Mn Can Cause Neuronal Injury



6. Consistent with results from microarray analysis, protein level of the inflammatory mediator COX2 is enhanced by manganese exposure

Human astrocytes were treated with 0, 10, 50, or 200 mM MnCl2 for 7 days. Then protein extracts were prepared and subjected to Western blotting analysis. The PVDF membranes were probed with antibodies against COX2 and b-actin, respectively. Equal amounts of cellular proteins were loaded in every lane

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